low due to presence of 1 mM EGTA and no added Ca^{2+} in the intracellular solution. When $[Ca^{2+}]_i$ was adjusted to 10 μM in whole-cell patch clamp mode and in perforated patch clamp experiments in which $[Ca^{2+}]_i$ was near resting levels, NVP-1 had a small effect on the current (28% at 10uM). In perforated patch clamp experiments, when cells were incubated with BAPTA-AM (50 μM for 30 min) NVP-1 blocked the channel with an IC_{50}=7.2 μM. The inhibitory effect of amitriptyline was not dependent on $[Ca^{2+}]_i$ and blocked the channel with an IC50 value of 1.2 μM in whole cell or perforated patch clamp experiments.
Our data suggest that inhibition of Nav1.5 channels by NVP-1 is calcium dependent. It is proposed that interaction of NVP-1 with the channel involves the IQ-motif or CaMKII phosphorylation which is prevented in low $[Ca^{2+}]_i$. It is also possible that in low $[Ca^{2+}]_i$. NVP-1 has low affinity for binding or even can bind to EFL or IQ-motif instead of Ca^{2+}/CaM/CaMKII.

1654-Pos Board B424
Voltage-Gated Sodium Channel (Na_V) Pore-Only Proteins
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The general topology repeating in the Voltage-Gated Ion Channel (VGIC) superfamily members includes six-transmembrane α-helical segments in which the first four form a voltage-sensing domain and the last two form the pore domain (PD). Studies of potassium channels from the VGIC superfamily together with identification of voltage-sensor only proteins have suggested that the VSD and the PD can fold independently. Whether such transmembrane modularity is common to other VGIC superfamily members has remained untested. Here we show, using protein dissection, that the *Silicibacter pomeroyi* voltage-gated sodium channel (Na_VSp1) PD forms a stand-alone, ion selective pore (Na_VSp1p) that is tetrameric, α-helical, and forms functional, sodium-selective channels when reconstituted into lipid bilayers. Mutation of the Na_VSp1p selectivity filter from LESWSM to LDDWSD, creates a calcium-selective pore-only channel, Ca_VSp1p. We further show that production of PDs can be generalized by making pore-only proteins from two other extremophile Na_Vs: one from the hydrocarbon degrader *Alcanivorax borkumensis* (Na_VAb1p), and one from the arsenite oxidizer *Alkalilimnicola ehrlichei* (Na_VAe1p). Together, our data establish a family of active pore-only ion channels that should be excellent model systems for study of the factors that govern both sodium and calcium selectivity and permeability. Further, our findings suggest that similar dissection approaches may be applicable to a wide range of VGICs and, thus, serve as a means to simplify and accelerate biophysical, structural, and drug development efforts.

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Voltage Sensor Module in NaV1.4: S1-S3 Charges Regulate Fast Inactivation
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We investigated the role of charged amino acid residues in the extracellular (ENC) and intracellular (INC) clusters of S1-S3 segments for each of four domains in skeletal muscle sodium channel NaV1.4. We used charge reversing and substituting mutations to show that the extracellular cluster of negative charge in domains III and IV promotes fast inactivation and limits its recovery. E1051R (S1) and D1069K (S2) in domain II slowed entry of channels into fast inactivation and accelerated recovery; E1051D and D1069E each rescued wild type fast inactivation parameters. In domain IV, destabilization of the fast-inactivated state by E1373K (S1) was not rescued in the E1373N mutation, whereas charge-dependent destabilization of fast inactivation was observed with mutations at N1389 (S2). Mutations within the ENC of domains I and II produced lesser effects on fast inactivation, but did inhibit activation. Effects on charge reversing mutations within the INC included an increase in the probability of fast inactivation from closed states. Homology models of the voltage sensor module of each domain were constructed from alignment of sequences of hNaV1.4 to NaVAb, using Modeller. Homology models were constructed for S1 through S4-S5 linker regions, basing the models on the data inferred from the crystal structure of the bacterial sodium channel (Payandeh et al., 2011). The models for hNaV1.4 voltage sensor module in domains I to IV support the hypothesis that S1-S3 counter charges influence the movement of S4 during activation and may explain the effects of mutations in these segments to regulate fast inactivation in a mammalian sodium channel.

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Contributions of Conserved Negative Surface Potentials in S2 and S3 to Voltage-Gating of Sodium Channels
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The question of how S4 charges are stabilized in the hydrophobic membrane environment is of critical importance to understand the function of voltage-gated ion channels (VGICs). Besides lipids, proteinaceous interactions are thought to play an important role and, indeed, the electronegative surface potentials of aromatic side chains and the net negative charge of acidic side chains are well suited to fulfill this role. Interestingly, a single aromatic side chain in S2 and a number of acidic side chains in S2 and S3 are highly conserved among VGICs. These residues have been extensively studied in potassium channels but little is known about their role in sodium channels. Here we incorporate unnatural amino acids to probe their contributions to channel gating in the skeletal sodium channel isoform (Nav1.4). Replacing the S2 aromatic in domains I-IV with fluorinated Phe derivatives does not affect channel gating, ruling out a cation-pi interaction with S4 charges. Trp substitutions in this position lead to a significant right-shift in the GV in domain I only, highlighting not only the functional differences between Nav domains, but also sodium and potassium channels, as the latter show a dramatic left-shift in the GV when Trp is introduced at the equivalent position in S2. Furthermore, we replaced Glu side chains with the neutral isosteric Glu analog nitrohomolalanine (Nha). Our data show that neutralizing a negative charge in S2 near the extracellular end of S4 leads to a significant right-shift in the GV in domain I, while neutralizing negative charges in S2 and S3 near the intracellular end of S4 result in small or no shifts in the GV. Together, these results shed new light on our understanding of voltage-gating in sodium channels.

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Sodium Channel Voltage Sensor Movements Are Influenced by the Auxiliary β1 Subunit
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Auxiliary β1 subunits are integral membrane proteins that play important roles in modulating the trafficking and gating properties of Nav channels. For example, co-expression of β1 with rNav1.2a in Xenopus oocytes shifts both steady-state inactivation and voltage-activation relationships to more negative voltages, and produces a pronounced hastening of fast inactivation. We started investigating the mechanism through which β1 alters Nav channel gating by exploring the hypothesis that this subunit interacts with one or more of the four voltage-sensing domains in rNav1.2a. We began by examining the influence of β1 on rNav1.2a gating currents, and observed a pronounced shift to more negative voltages in the presence of the subunit, suggesting that β1 facilitates voltage sensor activation. Although gating current measurements provide a powerful tool to investigate overall rNav1.2a voltage sensor mobility, they represent the collective motion of all four voltage sensors. Thus, to identify the influence of β1 on individual voltage sensor movements, we are currently combining gating current recordings with site-specific fluorescence measurements. We have also begun to examine whether β1 influences the voltage-sensor pharmacology of rNav1.2a. Our initial experiments with scorpion and tarantula toxins suggest that the actions of these toxins on rNav1.2a are unaltered by co-expression with the β1 subunit. Finally, we are also exploring the influence of lipid modification on the effects of the rNav1.2a/β1 complex, and have observed that enzymatic removal of the choline head group of sphingomyelin (thereby leaving the ceramide-1-phosphate in the lipid membrane) slows fast inactivation of rNav1.2a/β1 as if no auxiliary subunit is present. We are in the process of assessing the influence of this lipid modification on the interaction between rNav1.2a and β1 by recording gating currents together with changes in fluorescence signals from labeled regions within the channel.

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Proton Modulation of Gating Currents in the Cardiac Voltage-Gated Sodium Channel, NaV1.5
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Low pH reduces single channel conductance and destabilizes the fast-inactivated state of the cardiac voltage-gated sodium channel (NaV1.5) by increasing both window and persistent currents (Jones et al., 2011, Biophys. J. 101(7)). Since fast inactivation is tightly coupled to NaV channel voltage sensor activation, we hypothesized that alterations in the kinetics of voltage